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The early primary immune response to adsorbed tetanus toxoid in man

A study of the influence of antigen concentration, carrier concentration, and sequence of dosage on the rate, extent, and persistence of the immune response to one and to two doses of toxoid

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A quantitative study was performed to determine the effect of toxoid concentration and aluminium salt concentration on the primary immune response (PIR) and the secondary response induced by tetanus toxoid in human volunteers. Four toxoid preparations having 5-fold differences in toxoid concentration, aluminium salt concentration, or both, were administered to four comparable groups of human volunteers. Antitoxin titres in the serum of each volunteer were determined at intervals. The PIR was found to be a function of the antigen concentration, the mineral concentration, and the interaction of both. The secondary response was a function of the antigen concentration; increase in mineral adjuvant concentration had no significant effect. The data suggested that the higher the post-secondary response, the slower the rate of decline over the ensuing 10 months. The distribution of primary responses at day 28 tended to be bimodal. The response to the best preparation suggested that a single-dose toxoid might be developed to immunize populations that may be difficult to retrieve for multiple injections.

The traditional 2- or 3-dose schedule for basic immunization has a number of drawbacks. It frequently requires more time than is available—as, for instance, when immunization is required prior to imminent travel. It inevitably results in a significant percentage of uncompleted immunizations; numerous studies (1, 6) have disclosed a progressively increasing number of failures to return for each successive injection. Finally, in many circumstances—especially in underdeveloped countries or areas—multiple-dose schedules present serious problems as regards cost, staff, and logistics.

The primary immune response (PIR), i.e., the response to the initial dose of an inactivated antigen—and in particular the early response, i.e., the response in the first weeks—have been the subject of relatively few studies in man. Apart from the practical importance of such studies for simplified immunization, a more precise understanding of the dynamics of the PIR in man would clarify a variety of theoretical questions such as: (*a*) the identity of the factors that determine the magnitude and duration of the PIR, (*b*) the way in which these factors influence the response to subsequent inoculations of the same antigen, (*c*) the roles of antigen concentration and of adjuvant concentration in the primary as compared with the secondary response, and (*d*) whether host factors are important in determining the pattern of these responses.

Many similar questions could be asked, and the answers—with regard to man—are few. Animal analogies are of limited value. Marked differences exist between animal species, so that animal studies could provide general guidelines but could not be expected to forecast reliably the effects of various factors on the PIR in man.

Tetanus toxoid is probably the antigen most exten-

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sively studied quantitatively in man. It represents a well-characterized antigen for immunological studies and its effects can be evaluated serologically, not only in classical immunochemical terms but also in biological terms, by measuring toxin-neutralizing antibodies. The importance of being able to distinguish between these two types of response has been reemphasized in the last few years with the studies of Robbins (32) and others, which suggest that the toxin-neutralizing potency of antitoxic antibody may vary greatly, depending upon the type of immunoglobulin produced and the stage of the immune response under observation.

Numerous investigators have furnished data—usually as a baseline for subsequent injections—on the mean tetanus antitoxin level observed about 4 weeks after a single injection of tetanus toxoid in man (Table 1). Most of the observations suggest that a single injection usually induced little if any circulating antibody by the time that the second dose was administered (17, 29, 16, 33, 39, 25, 38). Only a few observations disclosed mean antibody levels at 3–4 weeks that exceeded 0.01 AU/ml, the generally accepted " protective threshold " (26), sometimes by a significant margin (17, 16, 30, 37, 24). The studies reported here were designed to define quantitatively

Table 1. Tetanus antitoxin response 3–4 weeks after 1 injection of toxoid

Reference no.	No. of subjects	Toxoid Lf/dose	Aluminium salt		Titres
			Form	Al^{+++} mg/dose	
17	14	1			0.0018 [a]
17	11	5			0.027 [a]
29	23 [b]	?			< 0.005 [a]
29	35 [c]	?			0.01 [a]
38	9	7.5	Alum	0.13	± 0.0025
39	29	5	PO_4	0.67	0.0005
24	3	10	PO_4	0.9	0.025
37	15	20	$(OH)_3$	0.9	± 0.02
25	21	5	PO_4	0.55	< 0.001
16	92	0.4	PO_4	1.1	0.0003
16	100	2	PO_4	1.1	0.001
16	99	10	PO_4	1.1	0.056
17	17	1	PO_4	1.33	0.0038 [a]
17	8	5	PO_4	1.33	0.013 [a]
30	16 [b]	200 BU	" adsorbed "		0.014 [a]
30	16 [c]	200 BU	" adsorbed "		0.08
33, lot IA	100	12 [d]	$(OH)_3$	1	0.0026
33, lot B	73	12	$(OH)_3$	1	0.0006
33, lot C	83	12 [d]	$(OH)_3$	1	0.0039
33, lot D	90	12	$(OH)_3$	1	0.00081
33, lot IIA	36	12 [d]	$(OH)_3$	1	0.0037
33, lot B	39	12	$(OH)_3$	1	0.0029
33, lot C	38	12 [d]	$(OH)_3$	1	0.011
33, lot D	45	12	$(OH)_3$	1	0.00087

[a] At 3 weeks; all other titrations carried out at 4 weeks.
[b] Subcutaneous administration.
[c] Intramuscular administration.
[d] Combined tetanus and diphtheria toxoids.

the role of antigen concentration and mineral salt concentration in regulating the PIR.

MATERIALS AND METHODS

Subjects

The 150 nurses and auxiliary nurses who volunteered for the toxoid injections and blood samplings were all residents of Cali, Departamento del Valle, Colombia, who denied having received tetanus toxoid on any previous occasion. Each subject who remained with the study was given the necessary injections to complete conventional tetanus immunization at the time of the final blood sampling.

Antigens

Tetanus toxoid, lot LP279, prepared according to the modification by Latham et al. (20) of the method devised by Mueller & Miller (27) and partially purified by the ammonium sulfate method of Levine & Stone (21), contained 1356 Lf doses per mg of protein (non-dialysable) nitrogen. Four 1-litre batches were made up to contain the concentrations of toxoid and aluminium phosphate shown in Table 2 designated lots A, B, C, and D. The high concentration of aluminium phosphate is the maximum permitted by the US control authorities. The sterile concentrated toxoid was added to a solution of aluminium chloride and sodium acetate shortly after the addition of equivalent sterile sodium phosphate ($Na_3PO_4.12H_2O$) solution so that the toxoid protein was adsorbed onto the aluminium phosphate precipitate as it was forming (22). Thiomersal, 0.01 %, was added and each lot was tested for sterility, safety, and potency, filled in 10-ml vials and labelled with instructions to inject intramuscularly in a 0.5-ml dose.[a] Prior to use in the study, each lot was injected into 3 or 4 volunteers from the laboratory staff; all but 2 had received tetanus toxoid injections previously, so that the test group was weighted in favour of probable reactivity to tetanus toxoid. Local reactions were insignificant and no systemic reactions occurred.

[a] Although these toxoid preparations conformed to official minimum requirements, they differed somewhat in composition from the licensed products currently distributed by these laboratories. They were therefore handled as a "New Drug" and the study was assigned IND No. 195 by the Division of Biologics Standards of the National Institutes of Health. Potency tests were performed according to the official requirements of the US control authorities and also in terms of International Units. The mean unit values obtained (41) are shown in Table 2.

Table 2. Characteristics of toxoids used in the Cali study

Composition			
Toxoid sublot	Tetanus toxoid Lf/dose	$AlPO_4$ mg/dose	International units/ml
A ("low-low")	5	0.77	51
B ("high-low")	25	0.77	70
C ("low-high")	5	3.86[a]	117
D ("high-high")	25	3.86[a]	381

Acceptability in man		
Toxoid	Subject	Local reaction[c]
A	E.M.D.	0
	E.J.B.	0
	H.F.E.	±
B	M.W.[b]	0
	C.W.	0
	M.C.E.	+
	R.E.P.	+
C	S.L.[b]	0
	M.S.	0
	A.P.	0
D	A.M.	±
	G.E.	±
	J.L.	±

[a] Maximum permitted by the United States control authorities.

[b] First injection; all other subjects had received previous injections of tetanus toxoid.

[c] There were no systemic reactions. ± = barely discernible; + = small (< 2") with local tenderness but no significant discomfort.

Design of study

The 150 adult female nurses or auxiliary nurses were recruited from the Hospital San Isidro and the Hospital Universitario and were:

1. Questioned for assurance that they were fully cognizant of the nature of the study and their role in it, and asked to sign a statement of agreement to participate in the study.

2. Entered in the roster, with relevant notes on their medical and immunization history; the toxoid to be injected (lot A, B, C, or D), was then assigned by rotation.

3. Examined by a physician (who did not see their toxoid assignment) to ascertain the state of their general health and whether they had a raised temperature, enlarged lymph nodes, or other signs of any existing inflammatory process.

4. Bled for preinjection titre.

5. Injected with the assigned toxoid.

6. Examined " blind " again the next day and the 7th day for reactions.

7. Bled again on days 7, 14, 21, and 28.

8. Given a 2nd injection, on either day 28 or day 56, of the same toxoid they received on day 0, as part of the agreement to complete their immunization (except for 2 individuals who had marked local reactions following the 1st inoculation).

9. Bled on day 56 to determine either the 2-month residual titre (in subjects reinjected on that day) or the 1-month booster titre following a 2nd injection (in subjects receiving their 2nd injection on day 28).

10. Examined for reactions one day after the 2nd injection. (Reactions seen on the 7th day after the 1st injection were too few and too mild to warrant a similar examination after the second.)

11. Those who could be located 373–378 days from the 1st injection were bled again and given a reinforcing injection of a commercial tetanus toxoid.

Bleedings were occasionally 1 or even 2 days off schedule, e.g., 7% were on day 7, and 20% on day 28. Such minor departures from schedule are ignored in the text and tables. The number of subjects who provided useful information included in Table 4 totalled 124.

Reactions

Evidence of any untoward subjective or objective reaction was sought at 1 and 7 days after the 1st and 1 day after the 2nd injection by physical examination (including temperature) and questioning. In particular, the oral temperature and the palpability of axillary nodes were observed 1 day after injection and compared with the findings a day earlier, just prior to injection.

Bleeding

Venous blood was collected under vacuum, the serum was drawn off the next day and frozen, and batches of serum were despatched from Cali to the USA by courier, either direct to Boston or to New Orleans and thence by air express to Boston, where sera were stored at −5°C until used.

Titrations

The antitoxin level in each serum was approximated by preliminary indirect haemagglutination tests (23); antitoxin titrations were then performed using 2-fold dilutions of serum mixed with appropriate concentrations of tetanus toxin and injected into mice (9), using 2 mice per dilution. Parallel tests were performed with standard antitoxin. The lowest final concentration of antitoxin determined was 0.0025 AU/ml of serum. The reliability of the technique was validated by numerous repeat titrations and by the general consistency of the values obtained, including 5 or more sequential bleedings in 138 of the subjects.

For estimation of geometric means and standard errors of treatment groups, subjects having titres of less than 0.0025 AU/ml were assigned the next lower titre, 0.00125 AU/ml. At day 14 a preponderance of subjects were in this category, and consequently the standard errors were artificially low. It was therefore decided to exclude confidence limits that were narrower than those of the mean of all 24 treatment groups by more than 2 standard deviations (Table 4). Although the means themselves might be suspect on the same ground, they were included because these day-14 means retained the same rank order as those of the 5 subsequent test intervals, thus demonstrating consistency with the entire data.

Comparisons of the efficacy of the study preparations were made on the basis of Student's " *t* "-test for the significance of the differences of geometric mean titres of the subject groups given in Table 4. 95% confidence limits were determined as 2 standard errors of the mean. Measurable titres on day 0 or day 7 were regarded as evidence of prior antitetanus immunity, and on this basis 18 individuals were excluded from the analysis of the data. Of these, 13 had measurable tetanus antitoxin titres on day 0 and hence were ineligible for inclusion in a study of PIR. Five others, although showing no measurable titre on day 0, showed a steep rise in titre in the next 7–14 days, to levels far above those normally seen in the typical range of a primary immune response as observed by ourselves and others and summarized in Table 1. Although we could not exclude the possibility that these 5 subjects were exceptionally efficient primary responders, it seemed wise to consider them as latent secondary responders and hence to exclude them. Thus the analysis of the study was based on 792 titred serum samples from the remaining 132 subjects. Of the bleedings scheduled on days 14, 21, and 28, 40 were missed, 17 because of vacations, 3 because of illness, 4 because of lack of suitable veins, 11 because of refusal, and 5 for other reasons. However, the distribution of subjects bled in each group on each scheduled occasion remained essentially uniform (see Table 4), indicating that refusals or absences could not apparently be related to any untoward effect produced by any particular sublot of toxoid.

Table 3. Incidence of reactions at 24 hours after first and second injections (Group I)

Reaction	A		B		C		D	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
headache	4/30	1/17	3/30	3/27	3/33	1/19	6/32	1/21
malaise	8/30	2/17	4/30	5/27	6/33	2/19	4/32	3/21
chills	2/30	1/17	1/30	3/27	2/33	0/19	0/32	2/21
arm pain	10/30	4/17	10/30	15/27	23/33	9/19	22/32	16/21
temperature > 37°C	2/30	—	4/30	—	3/30	—	3/30	—
tenderness								
slight	0	1	2	4	2	3	4	2
moderate	0	0	0	0	1	1	1	2
marked	0	0	0	0	0	0	2	0

RESULTS AND DISCUSSION

Reactions

All but 7 subjects were seen 1 day after the 1st and 2nd injections and questioned or examined for malaise, headache, chills, arm pain, and local tenderness or swelling. Although " arm pain " was recorded (in response to direct questioning) in one-third of the subjects receiving toxoids A or B (low mineral carrier) and in two-thirds of those receiving lots C or D (high mineral carrier), it did not constitute a deterrent to acceptance of a second injection (Table 3). Similarly, " malaise " was reported in between 12% (lot D) and 25% (lot A) of subjects, without any discernible correlation with toxoid lot. Three of 18 subjects with evidence of prior tetanus toxoid injections showed tenderness, as compared with 11 out of 123 without any apparent prior contact with the antigen. The distribution of these reactions does not suggest that any of the 4 lots of toxoid was unduly reactive. Two subjects who had fairly severe tenderness were excused from further injections. Ten subjects had temperatures of 37.1–37.6°C; the rest had temperatures of 37°C or below when seen. The observations at 1 week disclosed very few signs, symptoms, or complaints (e.g., arm pain in 8, as compared with 65 when seen at 24 hours) and do not warrant detailed presentation.

Following the 2nd injection, symptoms and/or signs were generally fewer and milder and were correlated not with " alum " content but, to a limited extent, with antibody response following the first injection. In general, there were fewer complaints of all types except " arm pain " following the 2nd injection.

Results of serum titrations

The geometric mean titres are given in Table 4 for days 14, 21, 28, 56, and 375 [a] and are shown graphically in Fig. 1. The distribution of the 28-day titres for all subjects is shown in Fig. 2. The bimodal character of some of these distributions is noteworthy (see below).

The relatively high potency of lot D as compared with that of lot A is obvious (Fig. 1). Lots B and C were not statistically distinguishable in the primary phase but lot B was significantly superior in the secondary, indicating that antigen rather than adjuvant is important in the secondary response.

Protection against tetanus

The response can also be examined in terms of the proportion of subjects with antitoxin levels at or above the protective threshold. Table 5 shows that, in these terms too, the response to lot A is low, that to lot D is high, and the responses to lots B and C are intermediate. It is interesting to note, however, that in terms of any measurable antitoxin, one-half of the subjects receiving lot D show a measurable response by 14 days. Yet there is one virtual " non-responder " in the D group; this subject never exceeded 0.02 AU/ml prior to her second injection, and when bled next at 1 year had a titre of less than 0.0025 AU/ml. All other subjects bled at 1 year had titres in excess of 0.01 AU.

[a] In fact, days 373–378. The subgroups receiving their second injection at 28 and 56 days were combined, since after one year the mean titres of each subgroup were not significantly different.

Table 4. Group I. Geometric mean tetanus antitoxin titres in AU/ml at sampling intervals in groups of women receiving 4 different adsorbed tetanus toxoid preparations

Day	Lot A 5 Lf/dose 0.77 mg $AlPO_4$/dose	Lot B 25 Lf/dose 0.77 mg $AlPO_4$/dose	Lot C 5 Lf/dose 3.86 mg $AlPO_4$/dose	Lot D 25 Lf/dose 3.86 mg $AlPO_4$/dose
14	(28) 0.0018 [d]	(29) 0.0027 [d]	(29) 0.0021 [d]	(28) 0.0032 (71)
21	(29) 0.0026 (76)	(31) 0.0046 (66)	(32) 0.0043 (71)	(29) 0.014 (59)
28	(29) 0.0038 (66)	(32) 0.0085 (58)	(30) 0.0080 (57)	(31) 0.028 (58)
56 [a]	(12) 0.0067 (51)	(13) 0.015 (47)	(19) 0.012 (66)	(17) 0.032 (55)
56 [b]	(14) 0.17 (43)	(14) 0.55 (47)	(14) 0.23 (36)	(13) 0.70 (44)
375 [c]	(10) 0.057 (54)	(16) 0.29 (58)	(19) 0.12 (62)	(18) 0.40 (47)

Parentheses preceding the mean give number of subjects; those following give the 95 % confidence limits expressed as a percentage which, when multiplied by the mean, gives the lower limit, and when divided into the mean, gives the upper limit.

[a] Before 2nd injection.

[b] 8 days after 2nd injection.

[c] 10–11 months after 2nd injection.

[d] Excluded because of excessive numbers of responses below the detectable level. See "Methods" for basis of exclusion.

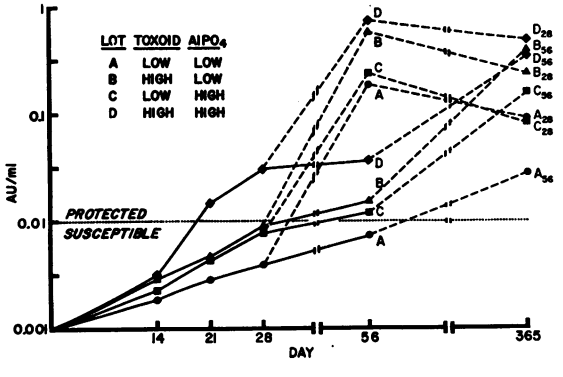

Fig. 1. Rate of serum tetanus antitoxin production in groups of women receiving 4 different adsorbed tetanus toxoid preparations. The horizontal dotted line represents the "threshold of protection". Continuous curves show responses to 1 dose, interrupted curves to 2 doses.

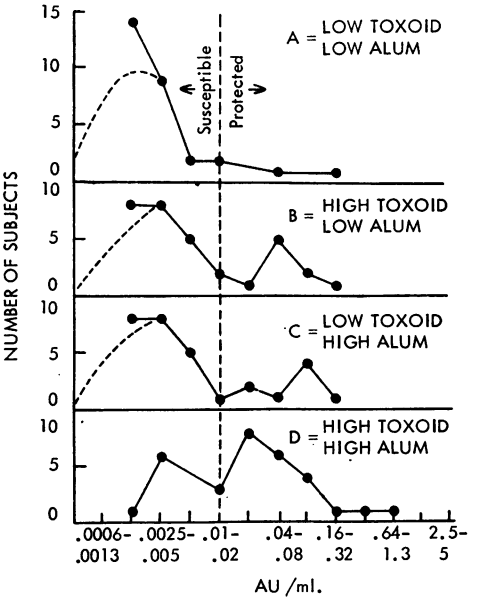

Fig. 2. Distribution of serum tetanus antitoxin titres by preparation 28 days after one injection. The interrupted lines represent presumptive "smoothing curves" approximating normal distributions for the low modes for which a number of responses were truncated, that is, below the detectable level.

The bimodal distribution of titres

Fig. 2 shows that the primary responses to preparations B, C, and D segregated the subjects into 2 classes at day 28. This bimodality was not seen at other intervals, perhaps because there were too few subjects, or because (prior to day 28) titres were too low for the second (higher) mode to have developed. A question of primary interest was the cause of this bimodal segregation. We do not consider the prior use of toxoid in these subjects to be an explanation of the bimodality, since, if this were the case, the first injection in such persons would have led to a secondary or booster-type response detectable on the 7th day. All subjects showing titres on the 7th day

Table 5. Number of subjects with titres below 0.01 [a] AU/ml.

Lot	Day 14	Day 14	Day 21	Day 28	Day 56 [b]	Day 56 [c]	Day 365 [d]
	< 0.0025 AU/ml	< 0.01 AU/ml					
A	27/28	28/28	27/29	25/29	8/12	0/14	0/10
B	18/29	28/29	23/31	21/32	4/13	0/14	0/16
C	24/29	28/29	26/32	21/30	8/19	1/14	0/19
D	15/28	26/28	10/29	7/31	1/17	0/13	1/18

[a] And, for day 14, < 0.0025 AU/ml.
[b] Before 2nd injection.
[c] 28 days after 2nd injection.
[d] 10–11 months after 2nd injection.

had already been excluded from the study. The possibility that growth hormone activity may play a major role in relation to the bimodal distribution of the PIR is now under study, as is the possibility that it is largely the result of genetic segregation.

High toxoid and low mineral concentration (lot B) compared with low toxoid and high mineral concentration (lot C)

It is apparent from Fig. 1 that lots B and C did not differ significantly from each other during the primary period but did differ from lots A and D. Following the second injection, B and C began to diverge, approaching D and A respectively. The P value for the significance of the superiority of B over C fell from about 0.85 on day 28 to 0.175 4 weeks after the 2nd injection and reached the significant value of 0.02 at one year. In those subjects who received secondary immunization on day 56, the P value diminished from 0.60 at that time to 0.08 at one year. This tendency of high toxoid (B) to give a better performance than high mineral (C) during the secondary response, in contrast to their near-equivalence during the primary, indicates that the mineral adjuvant makes little contribution to the secondary response. Expressed in more general terms, these findings are consistent with the general observation or impression recorded by many others in the past that a primary immune response is enhanced by adjuvant mechanisms much more readily than is the secondary or " booster " response (2, 12, 13). To give a specific example, it also makes understandable such findings as the failure of a mineral carrier to have an effect on the response of adults to influenza vaccine (5).

Effect of length of interval between the first and second injection on the response at 1 year

In an effort to test the widely reported favourable effect of longer injection intervals on the response, we gave one-half of the subjects their second injection after 4 weeks and the other half theirs after 8 weeks. However, only 63 of the 124 subjects could be retrieved for bleeding at one year and this small number did not yield significant response differences by interval. For this reason, the 2 subgroups are not separated at 1 year in Table 4.

Rate of fall-off in titre

There were insufficient subjects with matched bleedings in each subgroup to provide statistically significant data on the fall-off rate following a secondary response. However, the apparent rates of decline—66, 47, 48, and 42% respectively in subgroups A, B, C, and D—can be estimated from the values in the last two lines of Table 4. These figures are not inconsistent with the findings of Gottlieb et al. (10) and Peebles et al. (28) that, although there are possible exceptions (25), in general the higher the level of immunity attained the lower is its subsequent rate of decline.

A predictive equation for the primary response in man to a protein antigen adsorbed on mineral adjuvant

The day-28 responses of subjects receiving lots A and B define a primary dose–response regression on log Lf dose of toxoid at a constant level of mineral adjuvant. Those of the group C and D subjects similarly define another regression at a 5-fold higher mineral level. These 2 regressions have been drawn in Fig. 3 as straight lines connecting the mean responses of these pairs of subject groups, shown as 4 experi-

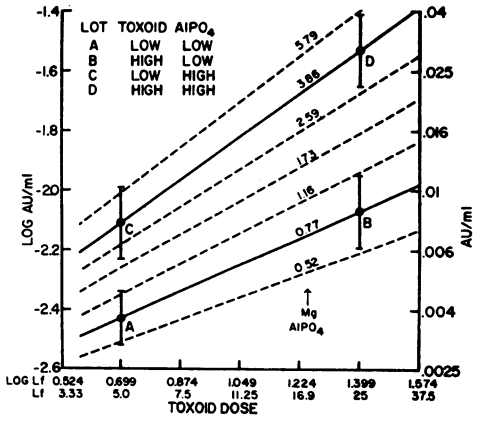

Fig. 3. Two-point primary logarithmic response regressions on toxoid dose at constant $AlPO_4$ at day 28. The 4 experimental points locate the mean (+ standard error) responses of about 30 subjects receiving each of the 4 lots A, B, C, and D. The interrupted lines illustrate the theoretical family of regressions postulated for successive 1.5-fold increments in $AlPO_4$ dose.

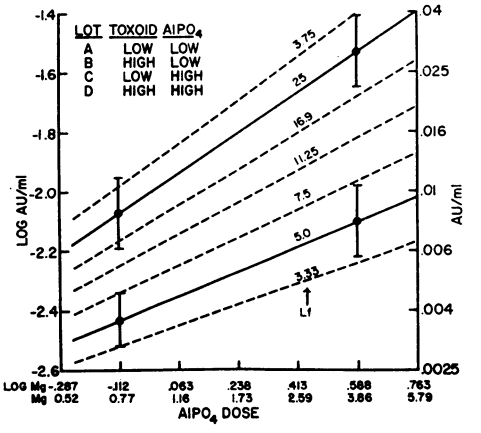

Fig. 4. Two-point primary logarithmic response regressions on $AlPO_4$ dose at constant toxoid on day 28. The 4 experimental points locate the mean (+ standard error) responses of about 30 subjects receiving each of the 4 lots A, B, C, and D. The interrupted lines illustrate the theoretical family of regressions postulated for successive 1.5-fold increments in toxoid dose.

mental points with the range of their standard errors. Line CD has a steeper slope than line AB, which represents one-fifth as much adjuvant. It has long been known that adjuvant toxoids often induce higher dose–response slopes than their fluid counterparts. This knowledge found its practical implementation in the necessity for the World Health Organization to establish two kinds of international standard for diphtheria and tetanus toxoids, one for fluid and one for adsorbed toxoids (8). Considerable data have since accumulated to show that the slopes also vary with the efficiency or with the concentration of the adsorbent as reflected in Fig. 3 (35). On the assumption that the slopes and intercepts of the dose–response lines are proportional to the logarithm of the mineral concentration, the interrupted theoretical regression lines have been drawn at 1.5-fold increments of adjuvant.

By analogy with Fig. 3, in which antigen is the independent variable, Fig. 4 shows the mineral concentration as variable, with antigen dose at constant levels. Line BD, representing a dose of 25 Lf of toxoid, has a steeper slope than line AC based on 5 Lf. The phenomenon of a slope difference depending on antigen concentration is much less well known than the variation of slope with mineral concentration, but was fully reported (without interpretation) by Schneider (35). He described 5 preparations of adsorbed tetanus toxoid having constant mineral and varying as follows in antigen concentration expressed as Lf per ml: 0.625, 2.5, 10, 40, and 160. The respective dose–response slopes as determined in mice were: 1.37, 1.73, 2.47, 2.21, and 3.06. A positive correlation is quite apparent. We calculated that 87% of the variance of these slopes could be accounted for by regression on log dose, thus justifying the theoretical (interrupted) lines shown in Fig. 4 and drawn at 1.5-fold increments of Lf dose.

The theoretical lines shown in Fig. 3 and 4 are based on the hypothesis outlined in the preceding two paragraphs that the slopes and intercepts of a dose–response line are directly proportional to the adjuvant content when " dose " refers to antigen, and to the antigen content when " dose " refers to adjuvant. This relationship provided the basis for the derivation of an equation that should predict the serum antibody titre for any combination of antigen and mineral dosage in this system 4 weeks after the injection. The general equation was derived as follows:

The equation of the dosage–response line may be written:

$$Z = bT + a \qquad (1)$$

where Z = the mean response in log AU per ml, T = the toxoid dose in log Lf, b is the slope, and a is the intercept.

On the assumption that the slope and intercept of this toxoid regression are directly proportional to the dose of aluminium phosphate,

$$b = b_1A + a_1 \quad (2)$$
$$a = b_2A + a_2 \quad (3)$$

where A = log $AlPO_4$ dose.

Substituting (2) and (3) in (1), we have the general equation

$$z = a_1T + b_2A + b_1AT + a_2$$

Applying the mean response data for day 28 of the present study, the coordinates of A (0.7, −2.43) and B (1.4, −2.07) give the equation of line AB:

$$Z = 0.514T - 2.79$$

Similarly C (0.7, −2.10) and D (1.4, −1.53) give

$$Z = 0.814T - 2.67$$

These 2 equations furnish the slope and intercept coordinates for evaluating the coefficients of equations (2) and (3) above, as follows: line AB (−0.112, 0.514), line CD (0.587, 0.814), giving (2) $b = 0.429A + 0.562$; and line AB (−0.112, −2.79), line CD (0.587, −2.67), giving (3) $a = 0.171A - 2.771$.

Substituting (2) and (3) in (1), we have

$$Z = 0.562T + 0.171A + 0.429AT - 2.771$$

This equation gives the day-28 mean responses to all 4 preparations as functions of the dose of toxoid and of adjuvant. Whereas we postulate that its general form may be applicable to primary immunization with adsorbed protein antigens in general, the values of the 4 coefficients obtained above would of course apply only to the specific conditions of our study. Among factors that will undoubtedly affect their value are: (*a*) the time interval after injection; (*b*) the quality of the immunizing agent; and (*c*) host factors, including age, sex, nutrition, and genetic constitution.

The possible effect of host factors is illustrated by comparing the results obtained with another group of subjects (7) who likewise received lot D. At 28 days the mean titre of this second group was 2.7-fold higher. This group included a high proportion of adolescent girls and was younger on average by 5 years. This age difference (and implicit difference in hormonal kinetics) may be only one of several host factors responsible for the 2.7-fold discrepancy when the above equation was applied to this younger group. When more is learnt about these factors, the information can then be inserted into the equation, as was done by Gottlieb et al. (11) in their analysis of the secondary response.

The form of this equation is relevant to our understanding of the primary response to the most practical types of immunizing agents. Prigge (31) expressed the potency of a toxoid in terms of an equation that took the simple form:

$$\text{potency units} = c\sqrt{Ag \times Adj}$$

where Ag represents antigen concentration and Adj represents adjuvant concentration. This equation, based on work with small animals, was still considered to be useful by at least 3 participants in an International Symposium on Adjuvants of Immunity held in 1966. Schneider (35) was troubled by the fact that, according to the equation, potency would vanish at zero adjuvant whereas in fact we know that plain toxoids do afford protection.[a] Schmidt (34), who was also disturbed, proposed a revised formula that would describe plain as well as adsorbed vaccines, but it was not supported by data.

Our equation, rather than expressing " potency " as an abstract entity, expresses the predicted response to a defined preparation. Its terms have certain important implications for the biometric assessment of vaccines. If the same standard could not be used for comparing plain and adsorbed antigens due to non-parallelism of dosage–response curves, do we not have the same problem with respect to preparations differing in mineral or antigen content? The answer is that such bioassays are indeed impossible if these disparities are great enough to demonstrate significant slope differences. In practice, however, the range of concentrations of these components is relatively narrow, making it unlikely that the theoretical slope difference will attain statistical significance. Nevertheless, in order to minimize unacceptable assays, it is incumbent upon the control authorities to select standards that are modal for these parameters.

These findings have further relevance for the mechanism of the immune response. Slope differences imply qualitative differences in the preparations and suggest that changes in the amount of either adjuvant or antigen will affect one or more of the factors involved in regulation of the immune response (19, 40). With further study, it should be

[a] In practice, however, the zero problem is obviated by a logarithmic transformation of the equation (or any similar equation such as the one we present).

possible to be more specific concerning the immunoregulatory mechanisms involved.

These findings demonstrate the major role of mineral adjuvant in determining the slope and height of the primary response, compared with its much smaller effect on the secondary response, and its role in the induction of a sustained residual antibody level following peak response. It must be recognized, however, that these relationships may not hold in ranges of concentration beyond those studied here; with excessively large doses of antigen, a primary response has been shown that was higher at the peak but fell thereafter more steeply than the responses following lesser antigenic stimuli (18, 36). Similarly, Holt (14) and Schmidt (34) have suggested that there is an optimum concentration for aluminium salts, above which the antibody level achieved falls rather than rises further. From the viewpoint of applied immunology as currently practised, however, this is an academic question, since our high dose of $AlPO_4$ will probably not be exceeded in practice, as it is, for instance, the maximum permitted by the US control authorities.

The possibility of defining the primary immune response quantitatively is only foreshadowed by the limited equation described herein; as Gottlieb et al. (11) have already noted, other empirical correlations such as age and sex can be detected in the analysis of the responses of relevant groups, and there are undoubtedly several additional major determinants of the immune response that still require study, such as malnutrition (3) and genetic determinants (4).

CONCLUSIONS

The early primary immune response to $AlPO_4$-adsorbed tetanus toxoid administered to human volunteer subjects was found to be a function of the antigen concentration, the adjuvant concentration, and their interaction. The empirical equation that fitted the mean responses of our subjects 28 days after a single injection of any combination of concentrations of antigen and adjuvant was

$$Z = 0.562T + 0.171A + 0.429TA - 2.771$$

where Z = mean log serum antitoxin titre, T = log toxoid dose (Lf), and A = log $AlPO_4$ dose (mg).

The secondary response was a function of the antigen concentration; increase in mineral concentration had little effect. It was shown that the higher the post-secondary response, the slower the rate of decline over the ensuing 10 months.

The distribution of primary responses at day 28 tended to be bimodal, segregating the subjects into high and low classes of immune responsiveness. This was not seen at other test intervals, perhaps because measurable titres, numbers of subjects, or both, were too small.

By a suitable choice of concentrations of antigen and mineral carrier, the primary immune response to one dose of tetanus toxoid could be markedly accelerated in time of appearance and rate of increase, giving a higher peak titre and prolonging the effective antibody level. Although the applicability of these findings to other antigens must be determined experimentally in each instance, it may reasonably be assumed that similar enhancement and acceleration of the primary immune response might be achieved with most other soluble protein antigens. These data hold out a promise that effective and acceptable immunization procedures based on a single primary injection of an optimally prepared toxoid—and possibly other antigens—may be feasible (7), although further studies on the role of age, sex, nutrition, and possibly other factors are required before any such procedure can be recommended for general use in tetanus immunization.

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RÉSUMÉ

RÉPONSE IMMUNITAIRE PRIMAIRE PRÉCOCE À L'ANATOXINE TÉTANIQUE ADSORBÉE CHEZ L'HOMME: ÉTUDE DE L'INFLUENCE DE LA CONCENTRATION DE L'ANTIGÈNE, DE LA TENEUR EN ADJUVANT ET DE L'INTERVALLE ENTRE LES INJECTIONS SUR LE TAUX, L'INTENSITÉ ET LA PERSISTANCE DES RÉPONSES IMMUNITAIRES APRÈS ADMINISTRATION D'UNE ET DE DEUX DOSES D'ANATOXINE

Les pratiques actuelles en matière de vaccination pourraient être améliorées, et probablement simplifiées, si l'on connaissait mieux les facteurs qui déterminent l'intensité et la durée de la réponse immunitaire. Dans la présente étude, on a voulu définir quantitativement le rôle de la concentration de l'antigène et de l'adjuvant sur les réponses immunitaires primaire (après la 1re dose) et secondaire (après la 2e dose) suscitées par un antigène largement utilisé, l'anatoxine tétanique adsorbée sur phosphate d'aluminium.

On a utilisé 4 préparations d'anatoxine du même lot, dosées de manière à contenir des quantités d'anatoxine, d'adjuvant, ou des deux, différant dans la proportion de 1 à 5. Chaque préparation a été administrée au hasard à environ 35 femmes adultes, volontaires, chez lesquelles les titres d'antitoxine ont été recherchés aux jours 0, 7, 14, 21, 28, 56 et 375 suivant la 1re injection. Une 2e injection a été donnée le 28e ou le 56e jour.

La réponse primaire a été influencée à la fois par la concentration d'antigène, la teneur en phosphate d'aluminium et par une action synergique des deux composants. On a pu exprimer mathématiquement l'importance relative de ces trois facteurs mesurée au 28e jour après la 1re injection. La réponse immunitaire à la 2e dose n'a guère été modifiée par la teneur en adjuvant, mais a varié modérément selon la quantité d'anatoxine injectée. Plus le titre d'antitoxine obtenu après la 2e injection était élevé, plus lent était son déclin pendant les 10 mois suivants. D'après l'analyse des titres d'antitoxine au 28e jour, il semble qu'on puisse classer les sujets vaccinés en « bons réacteurs » et en « réacteurs faibles ».

Les auteurs concluent que la réponse immunitaire suscitée par une dose unique de la préparation d'anatoxine la plus puissante laisse entrevoir la possibilité de recourir à ce schéma d'immunisation après un choix judicieux de la teneur en anatoxine et en adjuvant.

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